

CLAIMS

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1. A monomer polypeptide construct comprising at least one tetranectin trimerising structural element (TTSE) which is covalently linked to at least one heterologous moiety, said TTSE being capable of forming a stable triple alpha helical coiled coil complex with two other TTSEs, with the proviso that the heterologous moiety is different from any of the fusion proteins C11H6FXTN123, H6FXTN123, H6FXTN12, H6FXTN23, the sequences of which are shown in SEQ ID NOs: 24-27.

2. A monomer polypeptide construct comprising at least one tetranectin trimerising structural element (TTSE) which is covalently linked to at least one heterologous moiety, said TTSE being capable of forming a stable triple alpha helical coiled coil complex with two other TTSEs, said at least one heterologous moiety being one which does not exclusively facilitate expression and/or purification of the monomer polypeptide construct.

3. ^{The} A monomer polypeptide construct according to ^{claim 1} any of the preceding claims, wherein the heterologous moiety is selected from the group consisting of a ligand binding structure; a toxin; a detectable label; an in situ activatable substance; an enzyme; a radioactive moiety; a cytokine; a non-proteinaceous polymer such as a polymeric alkaloid, a polyalcohol, a polysaccharide, a lipid and a polyamine; a photo cross-linking agent; and a group facilitating conjugation of the monomer polypeptide construct to a target.

4. ^{The} A monomer polypeptide construct according to ^{claim 1} any of the preceding claims, which comprises 2 TTSEs which are covalently linked by a spacer moiety which allows both of the 2 TTSEs to take part in complex formation with a third TTSE not being part of the monomer polypeptide construct.

5. ^{The} A monomer polypeptide construct according to claim 4, wherein the spacer moiety has a length and a conformation which favours complex formation involving both of the two TTSEs which are covalently linked by the spacer moiety.

6. ^{The} A monomer polypeptide construct according to claim 4 or 5, wherein the spacer moiety is a polypeptide fragment.

7. ^{The} A monomer polypeptide construct according to ^{claim 1} any of claims 1-3, which comprises one single TTSE.

a 8. ^{The} ~~A~~ monomer polypeptide construct according to ^{Claim 1} ~~any of the preceding claims~~, wherein the TTSE is derived from human tetranectin, murine tetranectin, C-type lectin of bovine cartilage, or C-type lectin of shark cartilage.

a 5 9. ^{The} ~~A~~ monomer polypeptide construct according to claim 8, wherein the TTSE comprises a polypeptide sequence which has at least 68% sequence identity with the consensus sequence shown in Fig. 2.

a 10 ^{The} ~~A~~ monomer polypeptide construct according to claim 9, wherein the sequence identity with the consensus sequence is at least 75%, ~~such as at least 81%, at least 87%, or at least 92%.~~

a 11. ^{The} ~~A~~ monomer polypeptide construct according to ^{Claim 8} ~~any of claims 8-10~~, wherein at least one amino acid residue selected from the group consisting of amino acid residue nos. 6, 21, 22, 24, 25, 27, 28, 31, 32, 35, 39, 41, 42, is/are substituted by any non-helix breaking amino acid residue, the amino acid residue numbering referring to amino acid residues in SEQ ID NO: 7.

a 12. ^{The} ~~A~~ monomer polypeptide construct according to ^{Claim 1} ~~any of the preceding claims~~, wherein the at least one TTSE comprises a repeated heptad having the formula a-b-c-d-e-f-g (N to C), wherein a majority of the amino acids residues a and d are hydrophobic amino acids.

a 20 13. ^{The} ~~A~~ monomer polypeptide construct according to claim 12, wherein heptad is repeated 3 times and wherein the last occurrence of the heptad has a glutamine residue corresponding to residues a and d.

a 14. ^{The} ~~A~~ monomer polypeptide construct according to ^{Claim 1} ~~any of the preceding claims~~, wherein the at least one heterologous moiety is covalently linked to the TTSE via a peptide bond to the N- or C-terminus of the TTSE peptide chain, via a peptide bond to a side chain in the TTSE, via a bond to a cysteine residue, or when more than one heterologous moiety, combinations of these locations.

a 15. ^{The} ~~A~~ monomer polypeptide construct according to ^{Claim 1} ~~any of the preceding claims~~ which lacks any free amino and/or carboxy groups.

a 16. ^{The} ~~A~~ monomer polypeptide construct according to ^{Claim 1} ~~any of the preceding claims~~ which lacks a substantial part of the N-terminal region of tetranectin which is encoded by exon 1.

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a 17. ~~The~~ ^{claim 1} monomer polypeptide construct according to ~~any of the preceding claims~~ comprising two heterologous moieties which are linked via peptide bonds to the N- and C-terminus, respectively.

a 18. ~~The~~ ^{claim 1} monomer polypeptide construct according to ~~any of the preceding claims~~ which is constructed so as to disfavour formation of complexes between identical TTSEs.

Sub A3 19. An oligomer which is comprised of two monomer polypeptide constructs according to any of claims 1-18, and which comprises at least three TTSE's or a multipum of three TTSE's, or which is comprised of three monomer polypeptide constructs according to any of claims 1-3 or 7-18.

20. An oligomer according to claim 19 which is stable in the temperature range 50-70°C.

21. An oligomer according to claim 19 ~~or 20~~, which comprises at least one heterologous moiety which is positioned N-terminally to a TTSE and at least one heterologous moiety which is positioned C-terminally to a TTSE.

Sub C1 22. An oligomer according to claim 21, wherein the at least one heterologous moiety which is positioned N-terminally to a TTSE and the at least one heterologous moiety which is positioned C-terminally to a TTSE are part of the same monomeric polypeptide construct.

23. An oligomer according to claim 21, wherein the at least one heterologous moiety which is positioned N-terminally to a TTSE and the at least one heterologous moiety which is positioned C-terminally to a TTSE are part of two separate monomeric polypeptide constructs.

a 24. An oligomer according to ~~any of claims 19-23~~ ^{claim 19}, wherein each monomer polypeptide construct is designed so as to disfavour formation of trimers including two monomer polypeptide constructs having identical TTSEs.

a 25. A method of preparing a monomer polypeptide construct according to ~~any of claims 1-18~~ ^{claim 1}, the method comprising

- isolating the monomer polypeptide construct from a culture comprising a host cell which carries and expresses a nucleic acid fragment which encodes the monomer polypeptide construct, or

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- synthesizing, by means of chemical peptide synthesis, the monomer polypeptide construct and subsequently isolating the monomer polypeptide construct from the reaction mixture, or
- preparing a TTSE in a culture comprising a host cell which carries and expresses a nucleic acid fragment which encodes the TTSE, subsequently linking covalently at least one
- 5 heterologous moiety to the TTSE, and thereafter isolating the resulting monomer polypeptide construct, or
- synthesizing, by means of chemical peptide synthesis, a TTSE, subsequently linking
- 10 covalently at least one heterologous moiety to the TTSE, and thereafter the isolating the resulting monomer polypeptide construct from the reaction mixture,

and optionally subjecting the monomer polypeptide construct to further processing.

- 15 26. A method for preparing a dimeric oligomer ~~according to claim 19~~ which comprises

- admixing a monomer polypeptide construct according to ~~any of claims 1-18~~ ^{Claim 1} which includes two TTSEs (construct 1) with a monomer polypeptide construct according to ~~any of claims 1-18~~ ^{Claim 1} ~~3 or 7-18~~ which includes only one TTSE (construct 2),
- 20 - effecting the two TTSE's of construct 1 to complex with the TTSE of construct 2, and
- isolating the resulting dimer and optionally subjecting the dimer to further processing.

- 25 27. A method for preparing a trimeric oligomer ~~according to claim 19~~ which comprises

- admixing three monomer polypeptide constructs according to ~~any of claims 1-18~~ ^{Claim 1} with each other,
- 30 - effecting complex formation between one TTSE of each monomer polypeptide construct, and
- isolating the resulting trimer and optionally subjecting the trimeric oligomer to further processing.

- 35 28. A kit comprising

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- a
- 5 - a first package comprising at least one container means, each at least one container means containing a monomer polypeptide construct according to ~~any of claims 1-18~~ ^{Claim 1},
- a
- 5 - a second package comprising at least one container means, each at least one container means in the second package containing a monomer polypeptide construct according to ~~any of claims 1-18~~ ^{Claim 1}, the second package being different from the first package with respect to choice and/or number of monomer polypeptide constructs included therein, and optionally
- a
- 10 - a third package comprising at least one container means, each at least one container means in the third package containing a monomer polypeptide construct according to ~~any of claims 1-18~~ ^{Claim 1}, the second package being different from the first and second packages with respect to choice and/or number of monomer polypeptide constructs included therein.
- 15 29. A kit according to claim 28, wherein the at least one container means in each package contains mutually distinct monomer polypeptide constructs.
30. A kit according to claim 28 ~~or 29~~, wherein all container means comprised in the kit comprises mutually distinct polypeptide constructs.
- 20
31. A nucleic acid fragment in isolated form which encodes a TTSE as defined in ~~any of claims 1-18~~ ^{Claim 1} or which encodes the polypeptide part of a monomer polypeptide construct according to ~~any of claims 1-18~~ ^{Claim 1}, with the proviso that the nucleic acid fragment is different from one that encodes native members of the tetranectin family, and that the nucleic acid fragment is different from one that
- 25 encodes any of the fusion proteins C11H6FXTN123, H6FXTN123, H6FXTN12, H6FXTN23, the sequences of which are shown in SEQ ID NOs: 24-27.
32. A replicable vector which comprises a nucleic acid fragment according to claim 31.
- 30 33. A transformed host cell, which comprises a nucleic acid fragment according to claim 31 or a replicable vector according to claim 31.
34. Use of a monomer polypeptide construct according to any of claims 1-18 or to a an oligomer construct according to any of claims 19-24 for targeted gene therapy involving selective delivery of
- 35 the material for transfection or infection of the specific population of cells.

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35. The ~~use~~^{method} according to claim ~~34~~⁶⁵ wherein the at least one heterologous moiety comprises a moiety selected from a ligand binding structure such as a receptor molecule or the ligand binding part of a receptor molecule, and wherein the gene therapy involves the delivery of nucleic acids to the desired population of cells by use of a viral vector directed to cells displaying the artificial receptor complex corresponding to the heterologous moiety.

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36. The use of a monomer polypeptide construct according to any of claims 1-18 or to a an oligomer according to any of claims 19-24 as a component of a chimaeric product having low antigenicity in humans relative to formulations comprising on or more components of non-human origin.

37. The use of a monomer polypeptide construct according to any of claims 1-18 or to a an oligomer according to any of claims 19-24 as a vehicle for assembling antibody fragments into oligomeric or multivalent entities for generating chimeric artificial antibodies having preselected pharmacokinetic and/or pharmacodynamic properties.

38. The use of a monomer polypeptide construct according to any of claims 1-18 or to a an oligomer according to any of claims 19-24 for delivering an imaging or toxin-conjugated antibody to a tumor.

20 39. The use of a monomer polypeptide construct according to any of claims 1-18 or to a oligomer according to any of claims 19-24 as a vehicle delivering an substance to a target cell or tissue.

40. The use of a monomer polypeptide construct according to any of claims 1-18 or to a oligomer according to any of claims 19-24 for a labelled construct wherein the label is coupled to one or to of
25 the TTSE monomer units.

41. The use of a monomer polypeptide construct according to any of claims 1-18 or to a oligomer according to any of claims 19-24 for protein library technology, such as phage display technology.

30 42. The use according to claim 41 comprising a poly nucleotide molecule encoding one or more
 TTSE.

43. The use according to claim 41 comprising a vector encoding one or more TTSE.

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44. The use of a monomer polypeptide construct according to any of claims 1-18 or to a oligomer according to any of claims 19-24 for the preparation of a pharmaceutical composition.

45. The use according to any to claim 44, wherein the pharmaceutical composition further comprises
5 a pharmaceutically acceptable excipient.

46. The use according to claim 44 or 45 wherein the pharmaceutical composition is administered by a route selected from the group consisting of the intravenous route, the intraarterial route, the transmembrane route of the buccal, anal, vaginal or conjunctival tissue, the intranasal route, the
10 pulmonary route, the transdermal route, the intramuscular route, subcutaneous route, intrarectal route, inoculation into tissue such as a tumour, or by an implant.

47. The use according to any of claims 34 to 46
wherein the monomer polypeptide construct according to any of claims 1-18 or the oligomer
15 according to any of claims 19-24 is comprised in a liposome

48. A method for treating or preventing of a disease comprising administering to the subject in need thereof an effective amount of a pharmaceutical composition as defined in any of claims 44 and 45.

20 49. A method for treating or preventing a disease comprising administering to the subject in need thereof an effective amount of a relevant pharmaceutical coupled to a monomer polypeptide construct according to any of claims 1-18 or to a oligomer according to any of claims 19-24.

50. A method for targeted gene therapy comprising use of a monomer polypeptide construct
25 according to any of claims 1-19 or to a oligomer according to any of claims 19-24.

The method of Claim 45
51. ~~A method of human gene therapy comprising use of a monomer polypeptide construct according to any of claims 1-18 or to an oligomer according to any of claims 19-24 wherein at least one TTSE is modified with a membrane integrating or associating entity having affinity to the specific~~
30 ~~population of cells in the body relevant for the gene therapy.~~

The Claim 48 Composition
52. ~~A method according to any of claims 48 to 51 wherein the monomer polypeptide construct according to any of claims 1-18 or the oligomer according to any of claims 19-24 is administered by a route selected from the group consisting of the~~
35 ~~intravenous route, the intraarterial route, the transmembrane route of the buccal, anal og vaginal~~

a tissue, intranasal route, the pulmonary route, the ~~transdermal~~ route, intramuscular, subcutaneous, intratechal, the buccal, inoculation into tissue ~~such as a tumour~~, or by an implant.

a 53. A method for prevention and/or treating a disease, comprising administering to a mammal in
5 need thereof a prophylactically or therapeutically effective amount of a construct comprising the monomer polypeptide construct according to any of claims 1-18 or the oligomer according to any of claims 19-24.

Sub-A6 7 10 54. A method for diagnosis comprising a construct comprising the monomer polypeptide construct according to any of claims 1-18 or the oligomer according to any of claims 19-24 together with a diagnosing component coupled thereon.

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